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Effects of Nisin and Lysozyme on Growth Inhibition and Biofilm Formation Capacity of *Staphylococcus aureus* Strains Isolated from Raw Milk and Cheese Samples

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ABSTRACT

Effects of nisin and lysozyme on growth inhibition and biofilm formation capacity of 25 *Staphylococcus aureus* strains isolated from raw milk (13 strains) and cheese (12 strains) were studied. Nisin was tested at concentrations between 0.5 and 25 μg/ml; the growth of all strains was inhibited at 25 μg/ml, but the resistances of strains showed a great variation at lower nisin concentrations. In contrast, lysozyme tested at concentrations up to 5.0 mg/ml showed no inhibition on the growth of strains. Nisin used at the growth inhibitory concentration prevented the biofilm formation of strains, but strains continued biofilm formation at subinhibitory nisin concentrations. Lysozyme did not affect the biofilm formation of 19 of the strains, but it caused a considerable activation in the biofilm formation capacity of six strains. Twelve of the strains contained both biofilm-related protease genes (*sspA, sspB,* and *aur*) and active proteases; eight of these strains were nisin resistant. These results suggest a potential risk of *S. aureus* growth and biofilm formation when lysozyme is used in the biopreservation of dairy products. Nisin can be used to control growth and biofilm formation of foodborne *S. aureus,* unless resistance against this biopreservative develops.

There is growing interest in the use of biopreservation methods that employ natural antimicrobial compounds. Nisin, a well-known bacteriocin obtained from lactic acid bacteria, and lysozyme, an antimicrobial enzyme produced from hen egg white, are among the most promising candidates for use in the biopreservation of food *(13)*. The antimicrobial effect of nisin is due to its cationic nature, which helps it interact with anionic phospholipids at bacterial surfaces to form pores and to dissipate proton motive forces at the bacterial membrane, whereas lysozyme hydrolyzes the peptidoglycan (PG) layer at the bacterial cell walls *(2, 5, 36)*. Both nisin and lysozyme are effective against different gram-positive bacteria, but they are ineffective against gram-negative bacteria because of the protective lipopolysaccharide layer surrounding their PG layer at the cell walls.

In recent years, the effects of adding nisin and lysozyme to a variety of foods or incorporating them into plastic or biodegradable films intended for antimicrobial food packaging have been extensively studied *(43)*. The dairy industry was one of the first to be interested in using these generally recognized as safe substances. In fact, nisin and lysozyme are currently used effectively in cheeses as an alternative to nitrates to prevent late blowing caused by *Clostridium tyrobutyricum (8, 38)*. Nisin is also used in various cheese and milk products to inhibit gram-positive pathogenic bacteria, including *Clostridium botulinum, Listeria monocytogenes,* and *Staphylococcus aureus (13, 31, 35, 37)*.

S. aureus is a common cause of confirmed food poisoning and gastroenteritis resulting from the consumption of contaminated food *(24)*. The poisoning caused by this bacterium is frequently associated with raw milk and traditional cheeses made from unpasteurized milk, since the breasts of dairy cows can be a reservoir of enterotoxigenic *S. aureus* strains, a significant cause of mastitis *(18, 29, 31)*. One problem in controlling risks caused by *S. aureus* comes from its capacity to produce biofilms formed by an extracellular polysaccharide matrix and biofilm-associated proteins (BAPs) *(11)*. Because the biofilm formed by the bacteria increases its resistance to mechanical cleaning and disinfectants, the bacteria can spread into different parts of processing environments and subsequently contaminate food *(7, 9)*. Thus, cheese from heat-treated milk and whey might also carry significant risks unless processing equipment is decontaminated effectively and unless the curd obtained postheating is handled and stored properly *(21, 37)*. Biofilm is also an important virulence factor because it protects bacteria from opsonophagocytosis and antibiotics *(14, 25)*. BAP formation is a characteristic of mastitis-associated staphylococcal isolates *(12)*. Recent

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findings have supported the hypothesis that the phenotype of *S. aureus* can change from adhesive to invasive when it ceases protein-dependent biofilm formation and initiates mechanisms that cause biofilm degradation *(25)*. There is increasing evidence that the biofilm formation process and virulence of *S. aureus* are controlled by different types of extracellular proteases, such as V8 protease (SspA), staphopain B (SspB), and aureolysin (Aur), which are serine protease, cysteine protease, and metalloprotease secreted by staphylococci, respectively *(14)*. Martí et al. *(25)* showed a close relation of Aur and SspA overexpression and the degradation of BAP and cessation of biofilm formation in *S. aureus*. In addition, Shaw et al. *(39)* investigated the effects of mutations on *S. aureus* protease genes and reported the attenuation of the virulence of bacteria after insertional inactivation of *sspA* and *sspB* genes.

Studies have shown the nisin and lysozyme resistance of *S. aureus* strains *(3, 4, 20, 27)*. Recently, the lysozyme resistance of *S. aureus* has been shown to be related to the O-acylation of its peptidoglycan at the cell walls by an integral membrane protein, OatA *(3),* while resistance of *S. aureus* against nisin has been attributed to its reduced hydrophobicity and increased net positive charge following contact with this cationic peptide *(26)*. However, there are no studies related to variations in nisin and lysozyme resistances of foodborne *S. aureus* strains isolated from milk and dairy products. Moreover, there are few data available about the effects of these biopreservatives on the biofilm formation capacity of *S. aureus* and the possible roles of biofilm formation in its nisin and lysozyme resistance mechanisms. The primary objective of this study was to determine the effects of nisin and lysozyme on the growth inhibition and biofilm formation capacity of *S. aureus* strains isolated from raw milk and cheese samples. The genes controlling synthesis of proteases mediating protease activity and biofilm formation of bacteria were also investigated to better understand the possible causes of the varying biofilm formation capacity of the strains. The present study will provide a deeper elucidation of potential risks associated with foodborne *S. aureus* and the use of biopreservatives in dairy products.

MATERIALS AND METHODS

Biopreservatives. Lysozyme (≥40,000 U/mg of protein) from hen egg white and nisin from *Lactococcus lactis* (2.5%) were obtained from Sigma (St. Louis, MO).

Bacterial strains. The *S. aureus* strains (13 strains from raw milk samples and 12 strains from cheese samples) were previously isolated in Turkey and were characterized with molecular tests *(1)*.

Bacterial growth and biofilm formation. The effects of biopreservatives on *S. aureus* strains were analyzed in tryptic soy broth (TSB; Merck, Darmstadt, Germany) at pH 6.5 by spectrophotometric monitoring of turbidity formed by bacterial growth. The strains were grown on tryptic soy agar (TSA; Merck) at 37°C overnight, and the colonies were suspended in 10 ml of 0.9% (wt/vol) NaCl. Bacterial suspensions were adjusted to McFarland 0.5 ($\cong 5 \times 10^{10}$ CFU/ml) using a densitometer (Den-1, HVD Life Sciences, Austria) before being mixed with biopreservatives. One hundred eighty microliters of TSB containing nisin (at 0.5, 2.5, 12.5, and 25 μg/ml) or lysozyme (at 1, 2, 3, 4, and 5 mg/ml) and 20 μl of test strain were then mixed into a flat-bottom 96-well plate. The plate was incubated at 37°C for 24 h within the constant temperature plate holder of a microplate reader (Varioskan Flash, Thermo, Finland), and absorbance of well contents was determined at 600 nm every 15 min. The controls and cultures containing biopreservatives were tested in triplicate wells at each concentration, and averages of absorbance values versus time (minutes) were plotted to form growth curves. The following formula was used to calculate degree of inhibition: % inhibition = $100 - [(S1/S2) \times 100]$, where S1 is the slope of the best-fitting curve for a culture with biopreservatives and S2 is the slope of the best-fitting curve for the control (culture) at the linear growth phase of absorbance-time curves coming after lag periods. The lag period (minutes), which shows the delay in growth due to the effect of the biopreservative, was determined by subtracting the intercept of the best-fitting curve at the *x* axis for the linear growth phase of culture with a biopreservative from the intercept of the control.

At the end of the growth inhibition test (after 24 h of incubation at 37°C), the plate contents were tested for biofilm-forming capacity *(40)*. The plates were emptied and washed five times with 200 μl of phosphate-buffered saline (pH 7.0). The biofilm adhered at the surfaces of wells was then fixed with 200 μl of methanol and incubated for 15 min. The wells were then emptied and dried at 55°C for 1 h. The biofilm was stained with 200 μl of crystal violet for 5 min, and excess dye was washed off with water. The plate was dried, the absorbed crystal violet within wells was dissolved with 200 μl of 33% (vol/vol) glacial acetic acid, and optical densities of the well contents were measured at 590 nm using the microplate reader specified above. Absorbance values ≤0.1 were not considered to be color formed by biofilm since such low values were also formed by completely inhibited bacteria.

Detection of protease genes and active extracellular proteases. The presence of protease genes (*sspA, sspB,* and *aur*) was determined by PCR using the primers as described by Karlsson and Arvidson *(23)*. Bacterial genomic DNA isolation was carried out according to Sudagidan et al. *(42)*. The reactions were performed in 50 μl of reaction mixture containing 1.2 U of *Taq* DNA polymerase (Fermentas, Vilnius, Lithuania), 5 μl of 10× reaction buffer (750 mM Tris-HCl at pH 8.8 containing 200 mM $(NH_4)_2SO_4$, 0.1% [vol/vol] Tween 20, 1.5 mM $MgCl_2$), 10 μM of each of the primers (Metabion, Martinsried, Germany), 0.2 mM each of the four deoxynucleoside triphosphates (Fermentas), and 5 μl of the bacterial lysate as the DNA template. The PCR products were resolved in 1.5% (wt/vol) agarose gel electrophoresis in 1× TAE buffer (0.04 M Tris-acetate, 0.001 M EDTA). PCR experiments were done twice for each strain. Extracellular protease production of the strains was determined on the growth media containing casein as substrate *(41)*. The strains were spotted on casein agar plates and incubated at 37°C for at least 3 days. Clear zone formation around the spotted areas on agar after treatment with 5% (vol/vol) trichloroacetic acid showed the presence of the active extracellular proteases.

Statistical analysis. Statistical analysis was carried out by analysis of variance with a significance threshold of $P < 0.05$, as determined by Fisher's least significant difference test method using Minitab 15 software.

RESULTS AND DISCUSSION

Lysozyme tested at concentrations between 1 and 5 mg/ml did not show a considerable growth inhibitory effect on

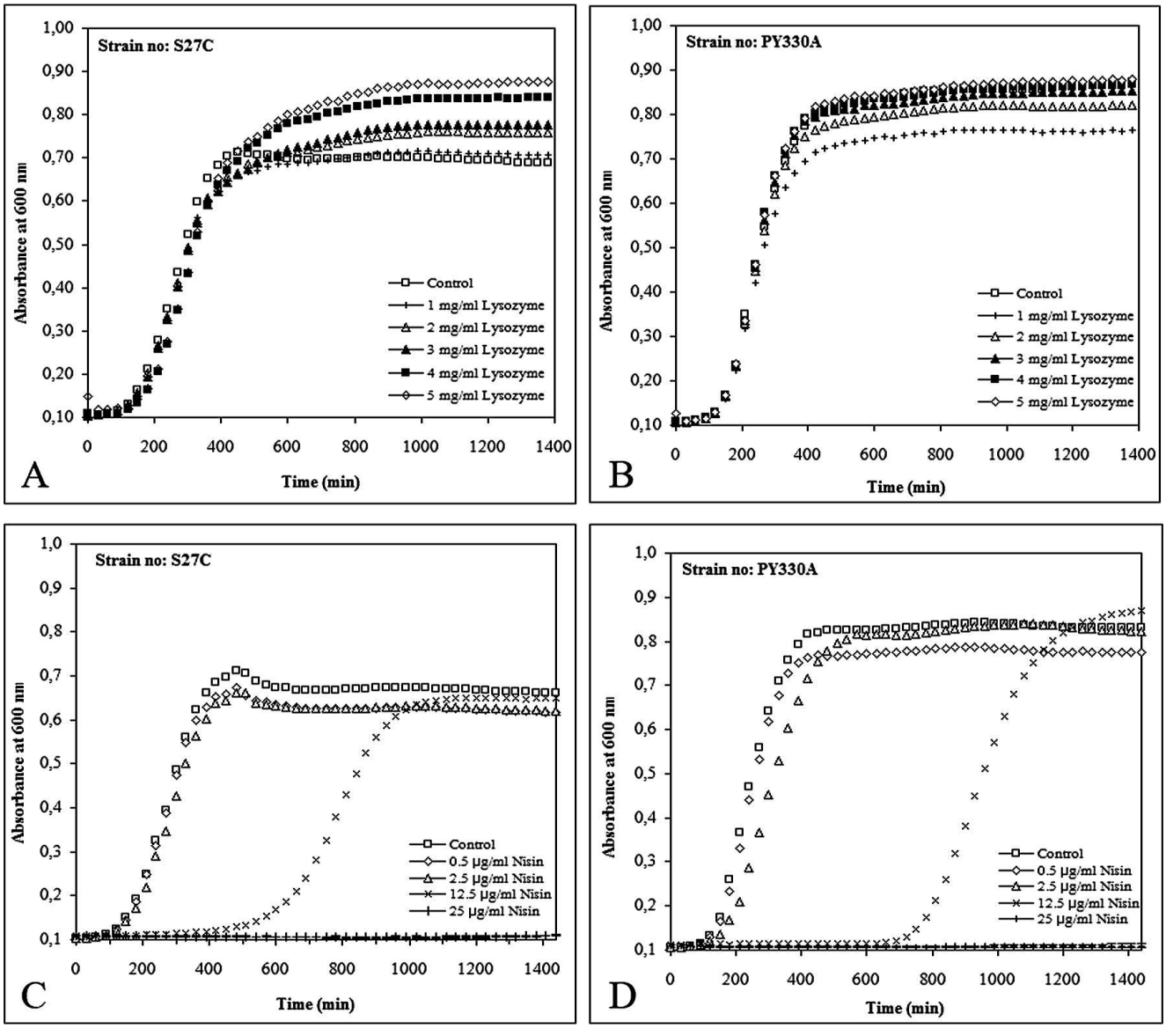

FIGURE 1. *Growth curves of selected* S. aureus *strains with different concentrations of lysozyme (A, B) or nisin (C, D).*

S. aureus strains. Also, no measurable lag periods were observed in the growth curves of strains in the presence of lysozyme. However, in nine strains isolated from raw milk samples (S170AY, S205Y, S267, S137AY, S158B, S292, S4BY, S133B, and S27C) and in four strains isolated from cheese samples (PY96B, PY1, PY2, and PY3), the presence of lysozyme prolonged the logarithmic growth phase and increased the absorbance values reached at the stationary phase (Fig. 1A). The absorbance values reached at the stationary phase of the indicated strains increased as the lysozyme concentration increased. However, more detailed actual plate counts are needed to report a limited growth activating effect of lysozyme on *S. aureus*. These changes in the growth curves were not observed in the remaining 12 strains. Instead, in the presence of lysozyme, these strains had growth curves similar to that of the control, or showed insignificant reductions in their growth rates (Fig. 1B). These results support the hypothesis that lysozyme resistance is an important virulence factor for *S. aureus (15)*.

Nisin at the 25-µg/ml concentration inhibited the growth of all *S. aureus* strains (Fig. 1C and 1D). However, the strains varied considerably in their resistance to nisin at subinhibitory concentrations (Table 1). The nisin resistances of strains were ranked by the degree of their growth inhibition at different nisin concentrations and by lag periods in their growth curves caused by this agent. In 2 of 13 strains isolated from raw milk (S170AY and S205Y), the bacteria showed high susceptibility to nisin, even at 0.5 µg of nisin per ml. Four strains (S267, S35A, S137AY, and S48A) showed moderate resistance and were inhibited at 12.5 µg of nisin per ml. The remaining seven strains, isolated from raw milk, were highly nisin resistant, and 25 µg of nisin per ml was needed to inhibit their growth completely. In contrast, 7 of 12 strains isolated from cheese samples showed moderate resistance against nisin and a nisin concentration of 12.5 µg/ml was needed for their complete inhibition. The only strain of cheese isolates highly susceptible to nisin was PY96B, which was inhibited at

TABLE 1. *Nisin resistances of* S. aureus *strains ranked by considering lag periods and inhibitions in their growth*[a]

Strain no.	0.5 μg of nisin/ml		2.5 μg of nisin/ml		12.5 μg of nisin/ml	
	Lag (min)	Inhibition (%)	Lag (min)	Inhibition (%)	Lag (min)	Inhibition (%)
Strains isolated from raw milk						
S170AY	—	−100	—	−100	—	−100
S205Y	—	−100	—	−100	—	−100
S267	85 ± 6.4 C[b]	−53 ± 3.9 E	394 ± 88 AB	−37 ± 13 HI	—	−100
S35A	34 ± 1.2 D	−5.4 ± 2.7 D	362 ± 17 B	−48 ± 5.6 I	—	−100
S137AY	20 ± 5.9 DE	−7.9 ± 5.2 D	78 ± 15 DE	−25 ± 3.9 FG	—	−100
S48A	0.8 ± 3.4 FG	−6.2 ± 1.5 D	31 ± 1.7 EF	−12 ± 2.7 BCDE	—	−100
S15A	4.2 ± 1.2 FG	−2.4 ± 0.0 BCD	53 ± 9.1 DEF	−25 ± 5.0 FGH	558 ± 23 B	−51 ± 17 BC
S158B	12 ± 1.8 EF	−2.7 ± 3.1 BCD	38 ± 3.2 EF	−13 ± 3.1 BCDE	583 ± 22 B	−50 ± 16 BC
S292	5.2 ± 1.4 FG	3.7 ± 1.6 ABC	16 ± 1.2 F	−0.93 ± 3.2 AB	531 ± 64 BC	−47 ± 6.0 ABC
S4By	6.0 ± 0.71 FG	1.8 ± 3.2 ABC	19 ± 0.82 EF	−6.4 ± 5.7 ABCDE	464 ± 9.2 CD	−48 ± 11 ABC
S133B	10 ± 2.4 EFG	4.9 ± 3.4 ABC	24 ± 3.5 EF	3.9 ± 3.4 A	405 ± 22 D	−58 ± 3.4 BC
S133A	7.9 ± 1.9 EFG	2.6 ± 2.6 ABC	23 ± 3.1 EF	−2.6 ± 2.6 ABC	435 ± 10 D	−34 ± 5.3 AB
S27C	—	−2.6 ± 2.2 BCD	12 ± 3.0 F	−2.6 ± 2.2 ABC	296 ± 11 E	−35 ± 3.9 AB
Strains isolated from cheese						
PY96B	207 ± 34 A	−23 ± 8.5 E	—	−100	—	−100
PY3	18 ± 2.6 DE	−2.8 ± 1.6 BCD	293 ± 15 C	−18 ± 13 EF	—	−100
PY192A	17 ± 2.2 DEF	0.0 ABCD	69 ± 12 DE	−14 ± 1.6 CDEF	—	−100
PY417A	5.8 ± 3.9 FG	−4.6 ± 4.2 CD	45 ± 7.8 DEF	−14 ± 3.2 CDEF	—	−100
PY280	6.6 ± 1.7 EFG	1.7 ± 3.0 ABC	36 ± 2.3 EF	−6.0 ± 1.5 ABCD	—	−100
PY31A	4.7 ± 1.6 FG	1.8 ± 1.5 ABC	16 ± 5.1 F	−11 ± 1.5 BCDE	—	−100
PY1	118 ± 3.0 B	−8.7 ± 4.4 D	431 ± 29 A	4.4 ± 4.4 A	—	−100
PY30C SARI	—	−1.0 ± 3.0 ABCD	12 ± 1.0 F	−3.0 ± 1.7 ABCD	—	−100
PY2	4.8 ± 2.8 FG	−1.1 ± 5.0 ABCD	26 ± 2.8 EF	2.2 ± 5.0 A	807 ± 45 A	−65 ± 15 C
PY134A	16 ± 0.9 DEF	8.2 ± 3.5 A	93 ± 10 D	−37 ± 1.8 GHI	561 ± 9.2 B	−44 ± 4.7 ABC
PY153C	5.5 ± 1.5 FG	6.0 ± 0.0 AB	15 ± 4.1 F	−6.9 ± 2.6 ABCDE	473 ± 65 CD	−51 ± 15 BC
PY330A	12 ± 1.3 EF	3.2 ± 0.0 ABC	42 ± 2.1 DEF	−15 ± 1.9 DEF	536 ± 43 BC	−24 ± 11 A

[a] All strains were inactivated at 25 μg of nisin per ml.
[b] Values with different letters in the same column are significantly different ($P < 0.05$).

2.5 μg of nisin per ml, while the remaining four strains (PY2, PY134A, PY153C, and PY330A) showed more nisin resistance and needed 25 μg of nisin per ml for complete inhibition.

In comparison with other studies related to nisin resistance of *S. aureus* strains, the MIC of 25 μg of nisin per ml determined for 11 of 25 strains in this study was higher than MICs reported for *S. aureus* strain 40 (0.54 μg of nisin per ml) and its nisin-resistant mutant (2.2 μg of nisin per ml) *(27)*. The nisin-resistant strains determined in this work also required higher MICs than 34 of 35 antibiotic-resistant *S. aureus* strains, which required a maximum MIC of 8.3 μg of nisin per ml (one strain required a MIC of >8.3 μg of nisin per ml) *(32)*. However, *S. aureus* CECT 4013 *(34)* and *S. aureus* Sa113 *(30),* requiring MICs of 25 and 23 μg of nisin per ml, respectively, showed similar resistance to the nisin-resistant strains in this study. In contrast, the strains tested in this work were considerably less nisin resistant than *S. aureus* Sa9R, a nisin-adapted strain requiring a MIC greater than 100 μg of nisin per ml *(26)*. An adaptation to nisin was also observed for *L. monocytogenes* mutants, with resistance to nisin up to 50 μg/ml (mutants were detected at frequencies of 10^{-6} to 10^{-8}) *(19)*. Peschel et al. *(30)* reported that the nisin resistances of *S. aureus* cells could be related to the amount of positively charged D-alanine esters in their cell wall teichoic acids and hypothesized that the increased positive charge at the bacterial cell walls was an important part of their resistance mechanism against cationic peptides including nisin. Recently, Martínez et al. *(26)* supported this hypothesis; they detected a net positive charge increase in a nisin-adapted *S. aureus* strain. However, these authors also detected a reduction in hydrophobicity of the nisin-adapted strain. There is also evidence that the nisin resistance of some *S. aureus* strains is related to a nisin-inactivating enzyme, nisinase *(6)*. Jarvis *(22)* showed the presence of nisin-inactivating enzymes in nisin-resistant *Bacillus* spp., but Grade et al. *(17)* attributed the nisin resistance of *Streptococcus thermophilus* INIA 463 to a thickening of its cell wall following nisin contact of this bacterium. These results clearly showed the presence of different nisin resistance mechanisms in bacteria and the need for further studies to investigate the existence or contribution of each mechanism in the nisin resistance of *S. aureus*. Further isolation and screening studies are also needed in dairy products to determine the frequency of isolation of nisin-resistant strains. According to the Codex Alimentarius, nisin can be applied to ripened cheese and whey protein cheese at a maximum level of 12 μg/g *(16)*. This nisin level seems

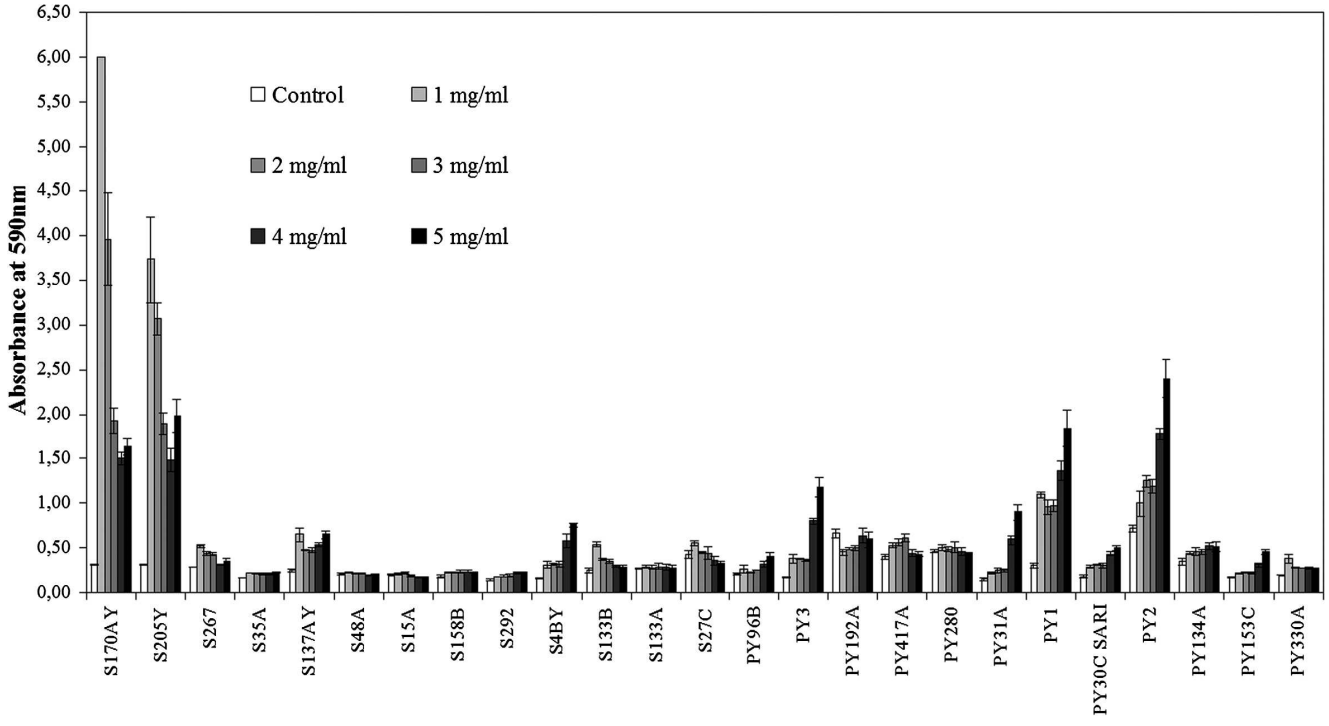

FIGURE 2. *Effect of lysozyme on biofilm formation of* S. aureus *strains.*

risky considering the nisin resistance of *S. aureus* strains studied in this work or reported in the literature. However, the good manufacturing practice level of 250 ppm (250 μg/g) in the finished processed cheese product determined by the British Standards Institution and approved by the U.S. Food and Drug Administration (FDA) was quite a safe nisin level to prevent poisoning caused by *S. aureus* in dairy products *(10)*.

The effects of lysozyme and nisin on biofilm formation capacity of 25 *S. aureus* strains are shown in Figures 2 and 3. Lysozyme either did not considerably affect or only slightly increased the biofilm formation capacity in 19 of 25 strains. In contrast, the enzyme caused a considerable activation in the biofilm formation capacity of six strains (PY1, PY2, PY3, PY31A, S170AY, and S205Y). Increased lysozyme concentration caused a considerable increase in

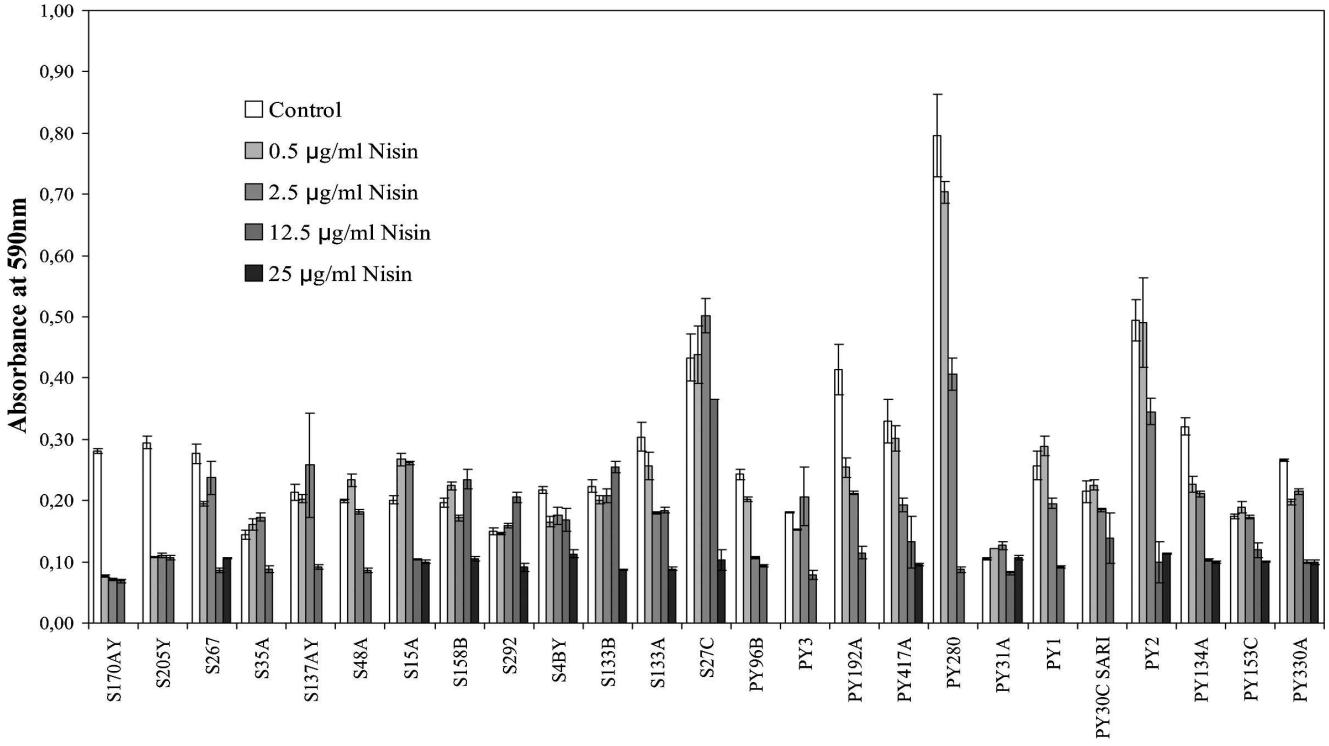

FIGURE 3. *Effect of nisin on biofilm formation of* S. aureus *strains.*

TABLE 2. *Biofilm-related protease genes and protease activity of* S. aureus *strains*

Strain no.	Protease genes			Protease activity[a]
	sspA	*sspB*	*aur*	
Strains isolated from raw milk				
S170AY	+	−	+	−
S205Y	+	−	+	−
S267	+	+	+	+
S35A	+	+	+	−
S137AY	+	+	+	−
S48A	+	+	+	−
S15A	+	+	+	+
S158B	+	+	+	+
S292	+	+	+	+
S4BY	+	+	+	−
S133B	+	+	+	+
S133A	+	+	+	+
S27C	+	−	−	+
Strains isolated from cheese				
PY96B	+	+	+	+
PY3	+	+	+	−
PY192A	+	+	+	−
PY417A	+	+	+	+
PY280	+	+	+	−
PY31A	+	+	+	+
PY1	+	+	+	−
PY30C SARI	+	+	+	+
PY2	+	+	+	−
PY134A	+	+	+	−
PY153C	+	+	+	+
PY330A	+	+	+	+

[a] +, presence of clearly identified zones (≥1 cm); −, absence of clearly identified zones (≥1 cm).

the biofilm formation capacity of PY1, PY2, PY3, and PY31A. In strains S170AY and S205Y, the highest amount of biofilm was formed with 1 mg/ml lysozyme, but the biofilm formation of these strains reduced as lysozyme concentration increased. The lysozyme did not cause a considerable change in the growth curve of PY31A, but in the remaining five strains the logarithmic growth phase was prolonged in the presence of lysozyme. These results suggest that biofilm formation capacity would be affected by the changed growth kinetics of bacteria in the presence of lysozyme. However, further studies are also needed to investigate if lysozyme activity at the bacterial surface triggers upregulation of gene synthesis for biofilm formation.

On the other hand, the presence of nisin at growth inhibitory concentrations reduced or inhibited the biofilm formation considerably (Fig. 3). Although most of the strains formed amounts of biofilm comparable to that in controls at subinhibitory nisin concentrations, no considerable activation occurred in biofilm formation capacity as was observed with lysozyme. No biofilm formation was detected for PY2 and PY280 strains; they showed the lowest nisin resistance and were inactivated even at 0.5 μg/ml nisin. We found no data in the literature related to biofilm formation capacity of different *S. aureus* strains in the presence of nisin. However, studies were conducted to incorporate nisin or immobilize it onto different supports to obtain materials having antimicrobial and biofilm inhibitory effects against bacteria, including *S. aureus (28, 33).*

The protease-encoding genes (*sspA, sspB,* and *aur*) and protease activity of strains were also investigated since specific extracellular proteases such as SspA, SspB, and Aur secreted by *S. aureus* might cause degradation of its protein-based biofilms and start its phenotypic change from adhesive to invasive. As seen in PCR results (Table 2), all strains contained the *sspA* gene. S170AY and S205Y strains lacked only *sspB,* whereas S27C, the most nisin-resistant strain isolated from raw milk, lacked genes of *aur* and *sspB*. On the other hand, the active proteases were detected mainly in nisin-resistant strains. The results indicated that six of seven nisin-resistant strains from raw milk samples, and two of four nisin-resistant strains from cheese samples, contained active extracellular proteases. In contrast, five strains (S170AY, S205Y, PY1, PY2, and PY3) that formed extensive amounts of biofilm in the presence of lysozyme lacked active proteases. Further studies using more strains are needed to report a potential correlation between the protease activity of *S. aureus* and its ability to form biofilms. However, these preliminary results suggest that the lack of active proteases prevented the degradation of BAP formed by these bacteria and that this improved their biofilm formation capacity.

In conclusion, the presence of lysozyme did not inhibit *S. aureus* strains isolated from raw milk and cheese samples and it increased the biofilm formation capacity of some strains. Nisin, at an appropriately high concentration, was effective to inhibit the growth of all strains completely, but it could not prevent biofilm formation at subinhibitory concentrations. Most of the nisin-resistant strains contained biofilm-related protease genes and active proteases, while most strains that formed extensive amounts of biofilm in the presence of lysozyme lacked active proteases. This study clearly showed the risk of biofilm formation by *S. aureus* in dairy products containing lysozyme as a biopreservative. The current good manufacturing practice limits of nisin in finished cheese products suggested by the FDA (250 μg/g) could be safe to inactivate *S. aureus* in dairy products. However, the maximum nisin limits suggested by the Food and Agriculture Organization and the World Health Organization (12 μg/g) may be risky for inactivation of the nisin-resistant strains. Further studies are needed to regularly monitor nisin-resistant *S. aureus* strains in dairy products and investigate the possible correlations among nisin resistance, biofilm formation capacity, and protease activity.

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